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                   enhanced
 NEWS  6  OCT 22   WPIDS, WPINDEX, and WPIX enhanced with Canadian PCT
                   Applications
 NEWS  7  OCT 24   CHEMLIST enhanced with intermediate list of
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                   and Japanese-language basic patents from 2004-present
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 NEWS 10  NOV 26   MEDLINE year-end processing temporarily halts
                   availability of new fully-indexed citations
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L1         531 (TEV) (4A) (PROTEASE OR PEPTIDASE OR PROTEINASE)

=> s l1 (P) (barrel or hinge)
L2           1 L1 (P) (BARREL OR HINGE)

=> d l2 bib ab

L2   ANSWER 1 OF 1  BIOSIS  COPYRIGHT (c) 2008 The Thomson Corporation  on STN
AN   2003:528567  BIOSIS
DN   PREV200300533286
TI   Structural and functional analysis of the PapC usher required for P pilus
     biogenesis.
AU   Kulkarni, R. P. [Reprint Author]; So, S. Shu Kin [Reprint Author]; Martin,
     C. [Reprint Author]; Thanassi, D. G. [Reprint Author]
CS   State University of New York at Stony Brook, Stony Brook, NY, USA
SO   Abstracts of the General Meeting of the American Society for Microbiology,
     (2003) Vol. 103, pp. B-179.
     http://www.asmusa.org/mtgsrc/generalmeeting.htm. cd-rom.
     Meeting Info.: 103rd American Society for Microbiology General Meeting.
     Washington, DC, USA. May 18-22, 2003. American Society for Microbiology.
     ISSN: 1060-2011 (ISSN print).
DT   Conference; (Meeting)
     Conference; Abstract; (Meeting Abstract)
LA   English
ED   Entered STN: 12 Nov 2003
     Last Updated on STN: 12 Nov 2003
AB   The PapC usher is a large integral outer membrane protein that, in
     conjunction with the periplasmic PapD chaperone, participates in the
     chaperone/usher pathway for biogenesis of P pili in Uropathogenic
     Escherichia coli.  Although the chaperone/usher pathway has been the focus
     of research for the past few decades, the details of the structure and the
     function of PapC have remained largely elusive.  The PapC monomer is
     predicted to contain 24 transmembrane beta-strands, forming a beta-
     barrel.  PapC monomers are thought to oligomerize to form
     ring-shaped complexes with central 2-3 nm diameter channels for secretion
     of pilus fibers to the cell surface.  To confirm the in vivo
     oligomerization of PapC, we used two different PapC constructs with either
     a hexahistidine or a c-myc epitope tag.  Co-purification of c-myc tagged
     PapC with the his-tagged version by nickel-affinity chromatography
     provided direct evidence for the oligomeric nature of PapC.  To
     experimentally map the topology of the PapC monomer, we generated random
     insertions of TEV protease cleavage sites.
     Accessibility of the protease cleavage sites for the action of TEV
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protease enabled us to define several surface exposed and periplasmic regions of PapC. The TEV insertion mutants were further analyzed for their effect on PapC function using whole bacterial expression, isolation of outer membranes, hemagglutination assays and pilus extractions. This functional analysis revealed that most of the TEV insertions did not affect the expression and outer membrane folding of PapC. However, the majority of the insertions caused a defect in pilus biogenesis. The C terminus of PapC has been implicated to function in the assembly and secretion of P pili. Using a charged-to-alanine site-directed mutagenesis approach, we were able to identify residues in the PapC C terminus important for pilus biogenesis. The conserved cysteine pair at the C terminus was also targeted for mutagenesis and was found to be an important structural motif for P pilus biogenesis.

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L4          73 L1 (P) (STRUCTURE)

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L5   ANSWER 10 OF 27     MEDLINE on STN                DUPLICATE 9
AN   2008188878     MEDLINE
DN   PubMed ID: 17985212
TI   Small-scale, semi-automated purification of eukaryotic proteins for
     structure determination.
AU   Frederick Ronnie O; Bergeman Lai; Blommel Paul G; Bailey Lucas J; McCoy
     Jason G; Song Jikui; Meske Louise; Bingman Craig A; Riters Megan; Dillon
     Nicholas A; Kunert John; Yoon Jung Whan; Lim Ahyoung; Cassidy Michael;
     Bunge Jason; Aceti David J; Primm John G; Markley John L; Phillips George
     N Jr; Fox Brian G
CS   The University of Wisconsin Center for Eukaryotic Structural Genomics and
     Department of Biochemistry, University of Wisconsin, Room 141B, 433
     Babcock Drive, Madison, WI 53706, USA.
NC   (5T32HG002760 (United States NHGRI)
     GM50853 (United States NIGMS)
     U54 GM074901 (United States NIGMS)
SO   Journal of structural and functional genomics, (2007 Dec) Vol. 8, No. 4,
     pp. 153-66.  Electronic Publication: 2007-11-06.
     Journal code: 101128185. ISSN: 1345-711X.
CY   Netherlands
DT   Journal; Article; (JOURNAL ARTICLE)
     (RESEARCH SUPPORT, N.I.H., EXTRAMURAL)
     (RESEARCH SUPPORT, NON-U.S. GOV'T)
     (RESEARCH SUPPORT, U.S. GOV'T, NON-P.H.S.)
LA   English
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FS Priority Journals
EM 200806
ED Entered STN: 20 Mar 2008
Last Updated on STN: 6 Jun 2008
Entered Medline: 5 Jun 2008
AB A simple approach that allows cost-effective automated purification of recombinant proteins in levels sufficient for functional characterization or structural studies is described. Studies with four human stem cell proteins, an engineered version of green fluorescent protein, and other proteins are included. The method combines an expression vector (pVP62K) that provides in vivo cleavage of an initial fusion protein, a factorial designed auto-induction medium that improves the performance of small-scale production, and rapid, automated metal affinity purification of His8-tagged proteins. For initial small-scale production screening, single colony transformants were grown overnight in 0.4 ml of auto-induction medium, produced proteins were purified using the Promega Maxwell 16, and purification results were analyzed by Caliper LC90 capillary electrophoresis. The yield of purified [U-15N]-His8-Tcl-1 was 7.5 microg/ml of culture medium, of purified [U-15N]-His8-GFP was 68 microg/ml, and of purified selenomethione-labeled AIA-GFP (His8 removed by treatment with TEV protease) was 172 microg/ml. The yield information obtained from a successful automated purification from 0.4 ml was used to inform the decision to scale-up for a second meso-scale (10-50 ml) cell growth and automated purification. 1H-15N NMR HSQC spectra of His8-Tcl-1 and of His8-GFP prepared from 50 ml cultures showed excellent chemical shift dispersion, consistent with well folded states in solution suitable for structure determination. Moreover, AIA-GFP obtained by proteolytic removal of the His8 tag was subjected to crystallization screening, and yielded crystals under several conditions. Single crystals were subsequently produced and optimized by the hanging drop method. The structure was solved by molecular replacement at a resolution of 1.7 A. This approach provides an efficient way to carry out several key target screening steps that are essential for successful operation of proteomics pipelines with eukaryotic proteins: examination of total expression, determination of proteolysis of fusion tags, quantification of the yield of purified protein, and suitability for structure determination.

L5 ANSWER 11 OF 27 MEDLINE on STN DUPLICATE 10
AN 2006754482 MEDLINE
DN PubMed ID: 17191617
TI Facile approach for constructing TEV insertions to probe protein structure in vivo.
AU Brown Elysa; Maloy Stanley
CS San Diego State University, San Diego, CA 92182-4614, USA.
SO BioTechniques, (2006 Dec) Vol. 41, No. 6, pp. 721-4.
Journal code: 8306785. ISSN: 0736-6205.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200701
ED Entered STN: 29 Dec 2006
Last Updated on STN: 5 Jan 2007
Entered Medline: 4 Jan 2007
AB The tobacco etch virus (TEV) protease has been used as a tool to examine protein structure in vivo. TEV cleavage sites (TEVcs) have been introduced via cloning into unique restriction sites or random transposon mutagenesis. We describe a facile, efficient method for introducing TEVcs at precise locations in a gene to test specific predictions about protein structure. The method uses the lamda

Red recombination system to construct seamless, in-frame insertions of the TEVcs at any desired location within an open reading frame (ORF). The system was tested using the multifunctional PutA protein Salmonella enterica sv. Typhimurium. The first step involved insertion of a chloramphenicol resistance (Cam(R)) cassette with a transcriptional terminator at the desired location. A second swap then replaces the Cam(R) insertion with the TEVcs. Placing a copy of the lac operon downstream of the putA gene provides a simple counterselection for replacement of the Cam(R) insertion and also provides a reporter gene for monitoring transcription of the mutated gene.

L5 ANSWER 12 OF 27 CAPLUS COPYRIGHT 2008 ACS on STN
AN 2005:297471 CAPLUS
DN 142:368696
TI Phage display for determining peptide ligand three dimension structure
IN Takahashi, Shigeo; Mizukoshi, Yumiko; Shimada, Kazuo
PA National Institute of Advanced Industrial Science and Technology, Japan; Japan Biological Informatics Consortium
SO Jpn. Kokai Tokkyo Koho, 18 pp.
CODEN: JKXXAF
DT Patent
LA Japanese
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI JP 2005087073	A	20050407	JP 2003-323441	20030916
JP 4184907	B2	20081119		
PRAI JP 2003-323441		20030916		

AB This invention provides a method for determining peptide ligand three dimension structure using phage display. The method consists of presenting the peptide ligand on surface of phage, cleaving the peptide from phage surface using protease, forming target-ligand complex in vitro and analyzing the the complex using NMR. The method provided in this invention can be used for anal. of mol. interaction.

L5 ANSWER 13 OF 27 MEDLINE on STN DUPLICATE 11
AN 2005153034 MEDLINE
DN PubMed ID: 15784745
TI Target-directed proteolysis at the ribosome.
AU Henrichs Tanja; Mikhaleva Natasha; Conz Charlotte; Deuerling Elke; Boyd Dana; Zelazny Adrian; Bibi Eitan; Ban Nenad; Ehrmann Michael
CS School of Biosciences, Cardiff University, Cardiff CF10 3US, UK.
SO Proceedings of the National Academy of Sciences of the United States of America, (2005 Mar 22) Vol. 102, No. 12, pp. 4246-51.
Journal code: 7505876. ISSN: 0027-8424.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LA English
FS Priority Journals
EM 200505
ED Entered STN: 24 Mar 2005
Last Updated on STN: 4 May 2005
Entered Medline: 3 May 2005
AB Target directed proteolysis allows specific processing of proteins in vivo. This method uses tobacco etch virus (TEV) NIa protease that recognizes a seven-residue consensus sequence. Because of its specificity, proteins engineered to contain a cleavage site are proteolysed, whereas other proteins remain unaffected. Therefore, this approach can be used to study the structure and function of target proteins in their natural environment within living cells. One

application is the conditional inactivation of essential proteins, which is based on the concept that a target containing a recognition site can be inactivated by coexpressed TEV protease. We have previously identified one site in the secretion factor SecA that tolerated a TEV protease site insert. Coexpression of TEV protease in the cytoplasm led to incomplete cleavage and a mild secretion defect. To improve the efficiency of proteolysis, TEV protease was attached to the ribosome. We show here that cleaving SecA under these conditions is one way of increasing the efficiency of target directed proteolysis. The implications of recruiting novel biological activities to ribosomes are discussed.

L5 ANSWER 14 OF 27 MEDLINE on STN DUPLICATE 12
AN 2005527853 MEDLINE
DN PubMed ID: 16204888
TI Three-dimensional structure determination of proteins related to human health in their functional context at The Israel Structural Proteomics Center (ISPC). This paper was presented at ICCBM10.
AU Albeck Shira; Burstein Yigal; Dym Orly; Jacobovitch Yossi; Levi Nurit; Meged Ran; Michael Yigal; Peleg Yoav; Prilusky Jaime; Schreiber Gideon; Silman Israel; Unger Tamar; Sussman Joel L
CS Department of Structural Biology, Weizmann Institute of Science, Rehovot 76100, Israel.
SO Acta crystallographica. Section D, Biological crystallography, (2005 Oct) Vol. 61, No. Pt 10, pp. 1364-72. Electronic Publication: 2005-09-28. Journal code: 9305878. ISSN: 0907-4449.
CY Denmark
DT Journal; Article; (JOURNAL ARTICLE) (RESEARCH SUPPORT, NON-U.S. GOV'T)
LA English
FS Priority Journals
EM 200511
ED Entered STN: 6 Oct 2005
Last Updated on STN: 15 Dec 2005
Entered Medline: 23 Nov 2005
AB The principal goal of the Israel Structural Proteomics Center (ISPC) is to determine the structures of proteins related to human health in their functional context. Emphasis is on the solution of structures of proteins complexed with their natural partner proteins and/or with DNA. To date, the ISPC has solved the structures of 14 proteins, including two protein complexes. It has adopted automated high-throughput (HTP) cloning and expression techniques and is now expressing in Escherichia coli, Pichia pastoris and baculovirus, and in a cell-free E. coli system. Protein expression in E. coli is the primary system of choice in which different parameters are tested in parallel. Much effort is being devoted to development of automated refolding of proteins expressed as inclusion bodies in E. coli. The current procedure utilizes tagged proteins from which the tag can subsequently be removed by TEV protease, thus permitting streamlined purification of a large number of samples. Robotic protein crystallization screens and optimization utilize both the batch method under oil and vapour diffusion. In order to record and organize the data accumulated by the ISPC, a laboratory information-management system (LIMS) has been developed which facilitates data monitoring and analysis. This permits optimization of conditions at all stages of protein production and structure determination. A set of bioinformatics tools, which are implemented in our LIMS, is utilized to analyze each target.

L5 ANSWER 15 OF 27 MEDLINE on STN DUPLICATE 13
AN 2005028473 MEDLINE
DN PubMed ID: 15654889

TI Comparison of the substrate specificity of two potyvirus proteases.
AU Tozser Jozsef; Tropea Joseph E; Cherry Scott; Bagossi Peter; Copeland Terry D; Wlodawer Alexander; Waugh David S
CS Department of Biochemistry and Molecular Biology, Research Center for Molecular Medicine, University of Debrecen, Debrecen, Hungary.. tozser@indi.biochem.dote.hu
SO The FEBS journal, (2005 Jan) Vol. 272, No. 2, pp. 514-23. Journal code: 101229646. ISSN: 1742-464X.
CY England: United Kingdom
DT (COMPARATIVE STUDY) Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200503
ED Entered STN: 19 Jan 2005 Last Updated on STN: 2 Mar 2005 Entered Medline: 1 Mar 2005
AB The substrate specificity of the nuclear inclusion protein a (NIa) proteolytic enzymes from two potyviruses, the tobacco etch virus (TEV) and tobacco vein mottling virus (TVMV), was compared using oligopeptide substrates. Mutations were introduced into TEV protease in an effort to identify key determinants of substrate specificity. The specificity of the mutant enzymes was assessed by using peptides with complementary substitutions. The crystal structure of TEV protease and a homology model of TVMV protease were used to interpret the kinetic data. A comparison of the two structures and the experimental data suggested that the differences in the specificity of the two enzymes may be mainly due to the variation in their S4 and S3 binding subsites. Two key residues predicted to be important for these differences were replaced in TEV protease with the corresponding residues of TVMV protease. Kinetic analyses of the mutants confirmed that these residues play a role in the specificity of the two enzymes. Additional residues in the substrate-binding subsites of TEV protease were also mutated in an effort to alter the specificity of the enzyme.

L5 ANSWER 16 OF 27 MEDLINE on STN DUPLICATE 14
AN 2005293963 MEDLINE
DN PubMed ID: 15919091
TI Crystal structure of tobacco etch virus protease shows the protein C terminus bound within the active site.
AU Nunn Christine M; Jeeves Mark; Cliff Matthew J; Urquhart Gillian T; George Roger R; Chao Luke H; Tscuchia Yugo; Djordjevic Snezana
CS Department of Biochemistry and Molecular Biology, University College London, Gower Street, London, WC1E 6BT, UK.
SO Journal of molecular biology, (2005 Jul 1) Vol. 350, No. 1, pp. 145-55. Journal code: 2985088R. ISSN: 0022-2836.
CY England: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE) (RESEARCH SUPPORT, NON-U.S. GOV'T)
LA English
FS Priority Journals
EM 200507
ED Entered STN: 8 Jun 2005 Last Updated on STN: 20 Jul 2005 Entered Medline: 19 Jul 2005
AB Tobacco etch virus (TEV) protease is a cysteine protease exhibiting stringent sequence specificity. The enzyme is widely used in biotechnology for the removal of the affinity tags from recombinant fusion proteins. Crystal structures of two TEV protease mutants as complexes with a substrate and a product

peptide provided the first insight into the mechanism of substrate specificity of this enzyme. We now report a 2.7A crystal structure of a full-length inactive C151A mutant protein crystallised in the absence of peptide. The structure reveals the C terminus of the protease bound to the active site. In addition, we determined dissociation constants of TEV protease substrate and product peptides using isothermal titration calorimetry for various forms of this enzyme. Data suggest that TEV protease could be inhibited by the peptide product of autolysis. Separate modes of recognition for native substrates and the site of TEV protease self-cleavage are proposed.

L5 ANSWER 17 OF 27 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN
AN 2006:159301 BIOSIS
DN PREV200600154761
TI Isolation of N-terminal extension of human lysyl-tRNA synthetase.
AU Keventzidis, Christina I. [Reprint Author]; Tsang, Pearl; Lenga, Natasha; Caperelli, Carol A.; Howell, Mike
CS St John Fisher Coll, Dept Chem, Rochester, NY 14618 USA
SO Abstracts of Papers American Chemical Society, (MAR 28 2004) Vol. 227, No. Part 1, pp. U434.
Meeting Info.: 227th National Meeting of the American-Chemical Society. Anaheim, CA, USA. March 28 -April 01, 2004. Amer Chem Soc.
CODEN: ACSRAL. ISSN: 0065-7727.
DT Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LA English
ED Entered STN: 9 Mar 2006
Last Updated on STN: 9 Mar 2006

L5 ANSWER 18 OF 27 CAPLUS COPYRIGHT 2008 ACS on STN
AN 2004:222531 CAPLUS
TI Isolation of N-terminal extension of human lysyl-tRNA synthetase
AU Keventzidis, Christina I.; Tsang, Pearl; Lenga, Natasha; Caperelli, Carol A.; Howell, Mike
CS Department of Chemistry, St. John Fisher College, Rochester, NY, 14618, USA
SO Abstracts of Papers, 227th ACS National Meeting, Anaheim, CA, United States, March 28-April 1, 2004 (2004), CHED-211 Publisher: American Chemical Society, Washington, D. C.
CODEN: 69FGKM
DT Conference; Meeting Abstract
LA English
AB Although the process of protein synthesis is generally well-understood, some important aspects of it remain poorly characterized. We have been interested in understanding the full function of human lysyl-tRNA synthetase (KRS) in vivo. In higher eukaryotic organisms, lysyl-tRNA synthetase has an N-terminal extension whose structure and function have yet to be determined To prepare significant amts. of human KRS (hKRS) for further study, a construct was produced using a pET-30a vector containing an external 6-His tag-S-tag-TEV protease cleavage site, followed by the amino acid sequence corresponding to the N-terminal domain of hKRS. Using gene expression techniques, the fusion protein was grown, isolated and purified. Upon removal of the 6-His tag-S-tag-TEV sequence via cleavage with TEV protease, the N-terminal extension was isolated. Such purification of the N-terminal extension of hKRS will enable further studies of the structure and function of this protein via spectroscopic techniques such as solution NMR, fluorescence and CD.

L5 ANSWER 19 OF 27 MEDLINE on STN DUPLICATE 15
AN 2004014818 MEDLINE
DN PubMed ID: 14711496
TI Recombinant proteins fused to thermostable partners can be purified by heat incubation.
AU de Marco Ario; Casatta Elisabetta; Savaresi Sara; Geerlof Arie
CS European Molecular Biology Laboratory, Heidelberg, Germany.. ademarco@embl-heidelberg.de
SO Journal of biotechnology, (2004 Jan 22) Vol. 107, No. 2, pp. 125-33.
Journal code: 8411927. ISSN: 0168-1656.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200408
ED Entered STN: 9 Jan 2004
Last Updated on STN: 17 Aug 2004
Entered Medline: 16 Aug 2004
AB We developed a protocol for the fast purification of small proteins and peptides using heat incubation as the first purification step. The proteins are expressed from a new bacterial expression vector (pETM-90) fused to the C-terminus of thermostable Ftr from Methanopyrus kandleri. The vector further contains a 6xHis-tag to allow immobilised metal ion affinity purification and a TEV protease cleavage site to enable the removal of the His-tag and fusion partner. Heat incubation induces the specific denaturation and precipitation of the Escherichia coli proteins but not of the thermostable fusion protein. Using the fusion construct and the heat incubation protocol a number of fusion proteins were purified to near homogeneity. The thermostability was ensured when Ftr had a molecular weight higher than twice the target protein. The obtained purification yields were similar and, in some cases, even higher than the ones obtained by affinity purification with the same Ftr-fusion proteins or the same target proteins fused to other often used partners such as NusA, GST, or DsbA. The protocol does not depend on a specific thermostable protein as was shown by the exchange of Ftr for M. kandleri Mtd. Purification by heat incubation is a fast and inexpensive alternative to chromatographic techniques, particularly suitable for the production of antigenic sequences for which the loss of native structure is not detrimental. We proved that it can be easily automated.

L5 ANSWER 20 OF 27 MEDLINE on STN DUPLICATE 16
AN 2004508265 MEDLINE
DN PubMed ID: 15477088
TI Efficient site-specific processing of fusion proteins by tobacco vein mottling virus protease in vivo and in vitro.
AU Nallamsetty Sreedevi; Kapust Rachel B; Tozser Jozsef; Cherry Scott; Tropea Joseph E; Copeland Terry D; Waugh David S
CS Macromolecular Crystallography Laboratory, Center for Cancer Research, National Cancer Institute at Frederick, P.O. Box B, Frederick, MD, USA.
SO Protein expression and purification, (2004 Nov) Vol. 38, No. 1, pp. 108-15.
Journal code: 9101496. ISSN: 1046-5928.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200507
ED Entered STN: 13 Oct 2004
Last Updated on STN: 27 Jul 2005
Entered Medline: 26 Jul 2005

AB Affinity tags are widely used as vehicles for the production of recombinant proteins. Yet, because of concerns about their potential to interfere with the activity or structure of proteins, it is almost always desirable to remove them from the target protein. The proteases that are most often used to cleave fusion proteins are factor Xa, enterokinase, and thrombin, yet the literature is replete with reports of fusion proteins that were cleaved by these proteases at locations other than the designed site. It is becoming increasingly evident that certain viral proteases have more stringent sequence specificity. These proteases adopt a trypsin-like fold but possess an unconventional catalytic triad in which Cys replaces Ser. The tobacco etch virus (TEV) protease is the best-characterized enzyme of this type. TEV protease cleaves the sequence ENLYFQG/S between QG or QS with high specificity. The tobacco vein mottling virus (TVMV) protease is a close relative of TEV protease with a distinct sequence specificity (ETVRFQG/S). We show that, like TEV protease, TVMV protease can be used to cleave fusion proteins with high specificity in vitro and in vivo. We compared the catalytic activity of the two enzymes as a function of temperature and ionic strength, using an MBP-NusG fusion protein as a model substrate. The behavior of TVMV protease was very similar to that of TEV protease. Its catalytic activity was greatest in the absence of NaCl, but diminished only threefold with increasing salt up to 200 mM. We found that the optimum temperatures of the two enzymes are nearly the same and that they differ only two-fold in catalytic efficiency, both at room temperature and 4 degrees C. Hence, TVMV protease may be a useful alternative to TEV protease when a recombinant protein happens to contain a sequence that is similar to a TEV protease recognition site or for protein expression strategies that involve the use of more than one protease.

L5 ANSWER 21 OF 27 MEDLINE on STN DUPLICATE 17
AN 2003439136 MEDLINE
DN PubMed ID: 14501138
TI Preliminary structure analysis of the DH/PH domains of leukemia-associated RhoGEF.
AU Kristelly Romana; Earnest Brett T; Krishnamoorthy Lakshmipriya; Tesmer John J G
CS Department of Chemistry and Biochemistry, The University of Texas at Austin, Austin, TX 78712, USA.
NC RR07707 (United States NCRR)
SO Acta crystallographica. Section D, Biological crystallography, (2003 Oct) Vol. 59, No. Pt 10, pp. 1859-62. Electronic Publication: 2003-09-19. Journal code: 9305878. ISSN: 0907-4449.
CY Denmark
DT Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
(RESEARCH SUPPORT, U.S. GOV'T, NON-P.H.S.)
(RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)
LA English
FS Priority Journals
EM 200405
ED Entered STN: 23 Sep 2003
Last Updated on STN: 26 May 2004
Entered Medline: 25 May 2004
AB Leukemia-associated RhoGEF (LARG) is a multidomain protein that relays signals from Galpha(12/13)-coupled heptahelical receptors to GTPases that regulate the cytoskeleton. To understand the molecular basis of LARG-mediated signal transduction, structural analysis of its DH/PH domains has been initiated. The LARG DH/PH domains have been overexpressed in Escherichia coli as a TEV protease

-cleavable fusion protein containing maltose-binding protein and a hexahistidine tag at the N- and C-termini, respectively. Crystals of the DH/PH domains were obtained (space group C2; unit-cell parameters a = 195.5, b = 46.0, c = 75.1 A, beta = 105.0 degrees) and xenon and NaBr derivatives were generated which should allow the structure to be determined by MIRAS.

L5 ANSWER 22 OF 27 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN
AN 2003:528567 BIOSIS
DN PREV200300533286
TI Structural and functional analysis of the PapC usher required for P pilus biogenesis.
AU Kulkarni, R. P. [Reprint Author]; So, S. Shu Kin [Reprint Author]; Martin, C. [Reprint Author]; Thanassi, D. G. [Reprint Author]
CS State University of New York at Stony Brook, Stony Brook, NY, USA
SO Abstracts of the General Meeting of the American Society for Microbiology, (2003) Vol. 103, pp. B-179.
http://www.asmusa.org/mtgsrc/generalmeeting.htm. cd-rom.
Meeting Info.: 103rd American Society for Microbiology General Meeting. Washington, DC, USA. May 18-22, 2003. American Society for Microbiology.
ISSN: 1060-2011 (ISSN print).
DT Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LA English
ED Entered STN: 12 Nov 2003
Last Updated on STN: 12 Nov 2003
AB The PapC usher is a large integral outer membrane protein that, in conjunction with the periplasmic PapD chaperone, participates in the chaperone/usher pathway for biogenesis of P pili in Uropathogenic Escherichia coli. Although the chaperone/usher pathway has been the focus of research for the past few decades, the details of the structure and the function of PapC have remained largely elusive. The PapC monomer is predicted to contain 24 transmembrane beta-strands, forming a beta-barrel. PapC monomers are thought to oligomerize to form ring-shaped complexes with central 2-3 nm diameter channels for secretion of pilus fibers to the cell surface. To confirm the in vivo oligomerization of PapC, we used two different PapC constructs with either a hexahistidine or a c-myc epitope tag. Co-purification of c-myc tagged PapC with the his-tagged version by nickel-affinity chromatography provided direct evidence for the oligomeric nature of PapC. To experimentally map the topology of the PapC monomer, we generated random insertions of TEV protease cleavage sites. Accessibility of the protease cleavage sites for the action of TEV protease enabled us to define several surface exposed and periplasmic regions of PapC. The TEV insertion mutants were further analyzed for their effect on PapC function using whole bacterial expression, isolation of outer membranes, hemagglutination assays and pilus extractions. This functional analysis revealed that most of the TEV insertions did not affect the expression and outer membrane folding of PapC. However, the majority of the insertions caused a defect in pilus biogenesis. The C terminus of PapC has been implicated to function in the assembly and secretion of P pili. Using a charged-to-alanine site-directed mutagenesis approach, we were able to identify residues in the PapC C terminus important for pilus biogenesis. The conserved cysteine pair at the C terminus was also targeted for mutagenesis and was found to be an important structural motif for P pilus biogenesis.

L5 ANSWER 23 OF 27 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN
AN 2003:528562 BIOSIS

DN PREV200300533281
TI Agrobacterium tumefaciens VirB9 protein, an outer membrane protein regulator of type IV secretion and piliation.
AU Jakubowski, S. J. [Reprint Author]; Christie, P. J. [Reprint Author]
CS Medical School, UT-Houston, Houston, TX, USA
SO Abstracts of the General Meeting of the American Society for Microbiology, (2003) Vol. 103, pp. B-175.
http://www.asmusa.org/mtgsrc/generalmeeting.htm. cd-rom.
Meeting Info.: 103rd American Society for Microbiology General Meeting. Washington, DC, USA. May 18-22, 2003. American Society for Microbiology.
ISSN: 1060-2011 (ISSN print).
DT Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LA English
ED Entered STN: 12 Nov 2003
Last Updated on STN: 12 Nov 2003
AB Biogenesis of a type IV secretion for translocation of T-DNA and effector proteins across the Agrobacterium tumefaciens cell envelope requires assembly of a disulfide-crosslinked heterodimer between the outer membrane-associated VirB7 lipoprotein and VirB9. Although VirB9 fractionated with the outer membrane in sucrose density gradients, the question of whether it spans the outer membrane has not been examined, and secondary structure predictions based on the algorithm of Schirmer and Cohen identified nine possible outer membrane spanning segments. Consistent with an outer membrane disposition, proteinase K treatment of whole cells of wild-type strain A348 degraded VirB9, but not periplasmic VirB proteins or the ChvE protein. For further structure - function studies aimed at exploring VirB9 topology and its role in substrate transfer and production of the T pilus, we constructed a series of in-frame XhoI restriction site insertions at 30-bp intervals along the length of virB9 (virB9.i2 alleles). For topological studies, a 31-residue epitope bearing a trypsin site, an epitope bearing a TEV protease site, and a FLAG epitope were introduced into the 16 permissive sites that were identified by complementation studies. Preliminary protease susceptibility and immunofluorescence studies support a proposal that VirB9 spans the outer membrane. Additionally, phenotypic studies of the 25 virB9.i2 alleles, as well as alleles bearing FLAG and i31 insertions, showed a general correlation between defects in piliation and substrate transfer. However, several VirB9.i2 mutations completely blocked substrate transfer without affecting biogenesis of the T pilus, or visa verse. These "uncoupling" mutations establish that VirB9 functions dually in biogenesis of the T pilus and assembly or function of a secretion machine for export across the outer membrane. Finally, several of the VirB9.i2 derivatives disrupted oligomerization of the bitopic inner membrane protein VirB10. The positions of these i2 insertions are consistent with previous work suggesting that the C-terminal region of VirB9 interacts with VirB10.

L5 ANSWER 24 OF 27 MEDLINE on STN DUPLICATE 18
AN 2002733862 MEDLINE
DN PubMed ID: 12377789
TI Structural basis for the substrate specificity of tobacco etch virus protease.
AU Phan Jason; Zdanov Alexander; Evdokimov Artem G; Tropea Joseph E; Peters Howard K 3rd; Kapust Rachel B; Li Mi; Wlodawer Alexander; Waugh David S
CS Macromolecular Crystallography Laboratory, Center for Cancer Research, NCI-Frederick, National Institutes of Health, Frederick, Maryland 21702-1201, USA.
NC N01-CO-56000 (United States NCI)
SO The Journal of biological chemistry, (2002 Dec 27) Vol. 277, No. 52, pp. 50564-72. Electronic Publication: 2002-10-10.

Journal code: 2985121R. ISSN: 0021-9258.
CY United States
DT (COMPARATIVE STUDY)
Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
(RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)
LA English
FS Priority Journals
OS PDB-1LVB; PDB-1LVM
EM 200302
ED Entered STN: 27 Dec 2002
Last Updated on STN: 28 Feb 2003
Entered Medline: 27 Feb 2003
AB Because of its stringent sequence specificity, the 3C-type protease from tobacco etch virus (TEV) is frequently used to remove affinity tags from recombinant proteins. It is unclear, however, exactly how TEV protease recognizes its substrates with such high selectivity. The crystal structures of two TEV protease mutants, inactive C151A and autolysis-resistant S219D, have now been solved at 2.2- and 1.8-A resolution as complexes with a substrate and product peptide, respectively. The enzyme does not appear to have been perturbed by the mutations in either structure, and the modes of binding of the product and substrate are virtually identical. Analysis of the protein-ligand interactions helps to delineate the structural determinants of substrate specificity and provides guidance for reengineering the enzyme to further improve its utility for biotechnological applications.

L5 ANSWER 25 OF 27 MEDLINE on STN DUPLICATE 19
AN 2001688800 MEDLINE
DN PubMed ID: 11708794
TI Production and characterization of the recombinant Sphingomonas chlorophenolica pentachlorophenol 4-monooxygenase.
AU Wang H; Tiirola M A; Puhakka J A; Kulomaa M S
CS Department of Biological and Environmental Science, University of Jyvaskyla, FIN-40351 Jyvaskyla, Finland.
SO Biochemical and biophysical research communications, (2001 Nov 23) Vol. 289, No. 1, pp. 161-6.
Journal code: 0372516. ISSN: 0006-291X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LA English
FS Priority Journals
EM 200112
ED Entered STN: 10 Dec 2001
Last Updated on STN: 23 Jan 2002
Entered Medline: 27 Dec 2001
AB Pentachlorophenol 4-monooxygenase (PCP4MO) from Sphingomonas chlorophenolica is a flavoprotein that hydroxylates PCP in the presence of NADPH and oxygen. In order to investigate the structure and function of active site, recombinant PCP4MO (rePCP4MO) was produced in Escherichia coli as a glutathione S-transferase (GST) fusion protein. Moreover, a tobacco etch virus (TEV) protease cleavage site (EKLYFQG) was introduced into GST-PCP4MO and a his-tagged TEV protease was employed. Hence, a two-step purification protocol was developed which allowed obtaining 15-20 mg of rePCP4MO from 1 L culture. The rePCP4MO revealed identity with native enzyme by SDS-PAGE and N-terminal sequence analyses. Furthermore, a polyclonal PCP4MO antibody was produced with GST-PCP4MO and purified by immunoaffinity chromatography, where both the native and recombinant forms of PCP4MO

showed interaction. However, rePCP4MO was identified as apoprotein with no evidence for a typical flavoprotein spectrum. The catalytic activity could be detected in the presence of FAD. The K(m) and V(max) values for PCP were 50 microM and 30 nmol/min/mg, respectively.

L5 ANSWER 26 OF 27 CAPLUS COPYRIGHT 2008 ACS on STN
AN 1998:239954 CAPLUS
DN 128:254392
OREF 128:50287a,50290a
TI A highly specific protease for the structure and functional analysis of proteins in vivo and in vitro
AU Ehrmann, Michael
CS Fakultaet Biologie, Univ. Konstanz, Konstanz, D-78434, Germany
SO BIOspektrum (1998), 4(2), 53-56
CODEN: BOSPFD; ISSN: 0947-0867
PB Spektrum Akademischer Verlag
DT Journal; General Review
LA German
AB A review with 5 refs. is given on the high-specific protease of the tobacco-etch-virus (TEV) which can be used for anal. of structure and function of proteins in vivo and in vitro including function of the TEV protease, insertion of recognition-sequences for the proteolytical cleavage into target proteins, proteolysis of the SecA protein, and genetic methods for investigations of structure and function of outer membrane proteins.

L5 ANSWER 27 OF 27 MEDLINE on STN DUPLICATE 20
AN 1989370313 MEDLINE
DN PubMed ID: 2475971
TI Characterization of the catalytic residues of the tobacco etch virus 49-kDa proteinase.
AU Dougherty W G; Parks T D; Cary S M; Bazan J F; Fletterick R J
CS Department of Microbiology, Oregon State University, Corvallis 97331-3804.
NC DK39304 (United States NIDDK)
SO Virology, (1989 Sep) Vol. 172, No. 1, pp. 302-10.
Journal code: 0110674. ISSN: 0042-6822.
CY United States
DT (COMPARATIVE STUDY)
Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
(RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)
LA English
FS Priority Journals
EM 198909
ED Entered STN: 9 Mar 1990
Last Updated on STN: 3 Feb 1997
Entered Medline: 29 Sep 1989
AB The 49-kDa proteinase of tobacco etch virus (TEV) cleaves the polyprotein derived from the TEV genomic RNA at five locations. Molecular genetic and biochemical analyses of the 49-kDa TEV proteinase were performed to test its homology to the cellular trypsin-like serine proteases. A cDNA fragment, containing the TEV 49-kDa proteinase gene and flanking sequences, was expressed in a cell-free transcription/translation system and resulted in the formation of a polyprotein precursor that underwent rapid self-processing. Site-directed mutagenesis was used to test the effect of altering individual 49-kDa amino acid residues on proteolysis. The data suggest that the catalytic triad of the TEV 49-kDa proteinase could be composed of the His234, Asp269, and Cys339. These findings are consistent with the hypothesis that the TEV

49-kDa proteinase is structurally similar to the trypsin-like family of serine proteinases with the substitution of Cys339 as the active site nucleophile. A structural model of the TEV 49-kDa proteinase proposes other virus-specific differences in the vicinity of the active site triad and substrate-binding pocket. The structure may explain the observed negligible effect of most cellular proteinase inhibitors on the activity of this viral proteinase.